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A Survey of *N*′-Nitrosonornicotine (NNN) and Total Water Content in Select Smokeless Tobacco Products Purchased in the United States in 2015

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Supporting Information

ABSTRACT: This investigation provides an updated survey measuring the levels of *N*′-nitrosonornicotine (NNN) and water content of a select number of smokeless tobacco products sold in the United States in 2015. A total of 34 smokeless tobacco products were collected and analyzed for NNN and water content using LC-MS/MS and GC-TCD, respectively. Smokeless tobacco products were chosen to obtain a representative sample of the different types of products on the U.S. market. These smokeless products represent 12 of the 25 top-selling smokeless tobacco products according to 2013 Nielsen net sales data while five of the smokeless tobacco products are of lower selling smokeless tobacco products. The NNN levels and the water content of the smokeless tobacco products were determined and compared to previous studies. Although the range of NNN levels found was broad for the examined smokeless tobacco products (0.64−12.0 μg/g dry weight), dry snuff had the highest levels of NNN observed (>5 μg/g dry weight). We observed a general decrease in NNN levels for the same six moist snuff products that were analyzed in 2004 compared to our current 2015 study. The water content of the smokeless tobacco products surveyed ranged from 3.92 to 54.8%.

KEYWORDS: *N*′-nitrosonornicotine (NNN), water content, smokeless tobacco products, LC-MS/MS, surveillance

INTRODUCTION

The Family Smoking Prevention and Tobacco Control Act (FSPTCA) amended the Federal Food, Drug, & Cosmetic (FD&C) Act and gave the FDA authority to regulate the manufacturing, distribution, and marketing of tobacco products in the United States. One of the most diverse categories of FDA-regulated tobacco products is smokeless tobacco products; these products are marketed in various forms including moist snuff, dry snuff, snus, loose leaf, plug, twist, and dissolvables. In 2010, an estimated 8.1 million people used smokeless tobacco products in the United States.[1] Smokeless tobacco products contain roughly 4000 distinct chemicals, composed of carcinogens and toxicants including tobacco-specific *N*′-nitrosamines (TSNAs).[2] *N*′-Nitrosonornicotine (NNN) is a TSNA formed by N-nitrosation of tobacco alkaloids, particularly during tobacco curing. NNN is one of the TSNAs classified as carcinogenic to humans by the International Agency on Cancer Research (IARC Group 1)[3] and is known to be one of the most abundant TSNAs found in smokeless tobacco products. NNN levels are generally lower in tobacco products under the following conditions: (1) products that use tobacco leaves that are cured under drier conditions, (2) products that use tobacco leaves that are air- or sun-cured as opposed to flue- or fire-cured, (3) products that contain minimum quantities of nitrogen-rich burley type of tobacco; (4) products that do not contain fermented tobacco, (5) products that have been heat-treated to deactivate microbial content, and (6) products that have been stored in dry conditions at or below room temperature.[4] Water content has been shown to play a role in NNN levels because tobacco products with high water activity support microorganism growth and microbial enzymes are involved in the reduction of nitrate to nitrite, which promotes nitrosation and NNN formation.[5] Differences in water content of smokeless tobacco products also affect product use and toxicant exposure. For example, Stepanov et al. reported that changes in snus products produced in the United States included larger pouch size and higher moisture content. They suggested that it is important to understand how these differences affect product use and toxicant exposure.[6]

There are relatively few published studies surveying the levels of NNN in smokeless tobacco products sold in various markets. They are primarily limited to United States and Swedish markets. Richter et al.[7] published NNN levels in 40 moist snuff products sold in the United States in 2004. NNN levels in Swedish smokeless tobacco products showed a significant reduction of NNN levels (from 1983 to 2002) due to the introduction of GOTHIATEK product standards for Swedish tobacco products.[8] GOTHIATEK is a quality control standard developed by Swedish Match (a smokeless tobacco product manufacturer) for the production of snus smokeless tobacco products. The standard applies not only to the manufacturing process (from the selection of raw materials and production techniques) but also to tolerances of the chemical composition of snus. The GOTHIATEK tolerance for TSNAs has changed

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over the years. In 2011, Rutqvist et al.[5] reported the tolerance for total TSNAs as 10 ppm dry weight, and, in 2012, Borgerding et al.[9] reported the same tolerance. However, as of 2016, the Swedish Match website reports the tolerance for NNN + NNK as 0.95 mg/kg on a wet weight basis.[10] NNN levels in U.S. smokeless products sold in 2006−2007[9] and 2007−2009[11] have also been published. The overall conclusions suggest that certain smokeless tobacco products (moist snuff and snus) contain lower TSNA levels than other smokeless tobacco products (dry snuff and chewing tobacco). In 2007, an IARC review reported worldwide NNN levels in smokeless tobacco.[4] Stepanov et al. published NNN levels in 12 snus products sold in the United States in 2010[12] and in 2011.[6] The authors suggested that TSNA levels in novel smokeless tobacco products (snus and dissolvables) can vary over time and can be controlled by the manufacturer. Stepanov and Hatsukami recently reported NNN levels, along with levels of other toxicants, in smokeless tobacco products sold in the United States in 2010 and 2011 and found highly variable levels of carcinogens and toxicants.[13] Hatsukami et al. recently reported the levels of TSNAs in U.S. moist snuff and snus products and demonstrated that exposure to TSNAs is dependent on TSNA levels and independent of pattern of use.[13,14]

As with all surveys of tobacco constituents, analytical method selection and detection is critical. Various chromatographic techniques have been used to determine TSNA levels in smokeless tobacco products. Stepanov et al.[15] analyzed various smokeless products by extracting with citrate−phosphate buffer using 5-methyl-*N*′-nitrosonornicotine (5-MeNNN) as an internal standard, followed by solid-phase extraction cleanup with final analysis by gas chromatography−thermal energy analyzer (GC-TEA). Stanfill et al.[16] used liquid chromatography−tandem mass spectrometry (LC-MS/MS) to quantitate TSNAs in rapé, a smokeless tobacco product native to South America. This method involves the use of ^{13}C-labeled TSNAs as an internal standard followed by extraction with aqueous ammonium acetate. CORESTA method No. 72[17] also involves the determination of TSNA in smokeless tobacco products by LC-MS/MS. The method involves the addition of deuterium labeled TSNAs to the sample followed by extraction with ammonium acetate, polytetrafluoroethylene (PTFE) filtration, and analysis by LC-MS/MS.

The water content of the smokeless products was also determined in many of these surveys. Many of these older survey market studies quantified moisture, or loss on drying, by measuring oven volatiles, not by measuring water content. Measurement of oven volatiles (by weighing the product before and after heating) may lead to inconsistent results compared to measuring water content directly since oven volatiles include not only water but also flavors and volatile decomposition products resulting from prolonged heating at 100 °C.[18] Measurement of water content by gas chromatography is a direct means of determining water content. Our current study is an up-to-date U.S. market study of NNN and water levels in smokeless products. The use of gas chromatography to quantify water in our study allows comparing NNN levels among smokeless tobacco products on a wet and dry weight basis.

MATERIALS AND METHODS

Tobacco Products. Thirty-four smokeless tobacco products from eight tobacco manufacturers, including small manufacturers,[19] were selected for analysis. The products included 18 moist snuff products, four snus products, four dry snuff products, and eight chewing tobacco products (four loose leaf, two plug, and two twist). Products were chosen to obtain a representative sample of the different types of smokeless products on the market. These smokeless products represent 12 of the 25 top selling smokeless tobacco products according to 2013 Nielsen net sales data while five of the smokeless tobacco products are of lower selling smokeless tobacco products. Many of the smokeless tobacco products surveyed contained characterizing flavors such as wintergreen, apple, cherry, mint, and frost. Smokeless tobacco products were purchased in mid-January 2015 in retail stores in Arkansas and Alabama. Samples were shipped at ambient temperature in shipping containers to Labstat International Inc. (Kitchener, Ontario, Canada) within seven days of purchase and received by the beginning of February 2015. Samples were stored at 4 °C between receipt and analysis. Labstat International Inc. performed all NNN and water analyses between March and April 2015. When available, seven containers of each smokeless tobacco product were obtained and one replicate from each container was analyzed. The average of these seven replicates was reported. For Stoker's Wintergreen Long Cut and Big Mountain Chewing Tobacco, only three and four containers, respectively, could be located in retail stores. For these two products, the seven replicates were taken from a single composite sample made by mixing the contents of all containers. For Camel Snus Frost, one container of Camel Snus Frost Large was tested and averaged with six containers of Camel Snus Frost. For pouched smokeless tobacco products, the sample preparation method involved grinding the pouch and analyzing it along with the tobacco.

NNN Analysis. NNN was analyzed by LC-MS/MS as previously described by Labstat International.[20] The ground products were extracted with ammonium acetate buffer and filtered, and the levels of NNN were quantified by LC-MS/MS using positive electrospray ionization and NNN-d_4 as an internal standard. Two transition pairs for nondeuterated NNN were used for quantification and confirmation. The ion pairs for NNN were 178/148 (quantifcation) and 178/120 (confirmation). The ion pair for NNN-d_4 was 182/152. The method has a limit of detection of 2.38 ng/g and a limit of quantification of 7.93 ng/g.

Water Content Analysis. Water content was quantified by gas chromatography with detection by thermal conductivity according to ISO method 16632:2013.[21] Water was extracted into a methanol solution, and isopropanol was used as an internal standard.

Products Purchased, Expiration/Sell-by Dates, and Locations. Label and purchase information such as brand name, name and address of retail establishment, date and time of purchase, UPCs, lot numbers, images, and expiration/sell-by dates for all products were recorded by the contractor tasked to obtain smokeless tobacco products at retail locations for this study and are available in the Supporting Information. Of the 34 products purchased in January 2015, 16 had no expiration or sell-by dates while 18 had an expiration or sell-by dates on the packaging. A majority of snus and moist snuff products had expiration/sell-by dates (75% snus and 83% moist snuff had expiration/sell-by dates), while dry snuff and chewing tobacco products did not have expiration/sell-by dates. Two products were sold after the sell-by or expiration dates (Timber Wolf Straight Long Cut and Hawken Wintergreen). Because our study was intended as a snapshot of smokeless tobacco products available for purchase by consumers, we included these products in the analysis. We originally intended to purchase all products from the state of Arkansas in January 2015 since that state had a high percentage of respondents to the Behavioral Risk Factor Surveillance System survey in 2009 and 2011−2013 who answered "every day" or "some days" to the question: "Do you currently use chewing tobacco, snuff, or snus [a small pouch of smokeless tobacco] every day, some days, or not at all?"[22] Due to low product availability in Arkansas, we expanded the search for products into Alabama. The products purchased in Albertville, Alabama, were Tops Sweet Snuff, Hawken Wintergreen, Silver Creek Cherry, King B Twist, and Mammoth Cave. The products purchased in Little Rock, Arkansas, were Stoker's Wintergreen Long Cut, Bowie Loose Leaf, Big Mountain, and Days O Work. The product

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purchased in Centre, Alabama, was Bruton Scotch Snuff. The 24 other products were purchased in Benton, Arkansas.

RESULTS AND DISCUSSION

NNN Results. The NNN data set is reported in Table 1 on a dry weight basis dependent on the water content. Figure 1A shows NNN concentrations as a function of percent water, grouped by product type, and Figure 1B shows NNN concentrations sorted by product type and colored accordingly. The data reveal that the range of NNN concentrations for all examined types of smokeless tobacco products was 0.635 to 12.0 μg/g on a dry weight basis. However, as shown in the figures below, higher or lower levels of water content do not necessarily have a direct correlation to higher or lower levels, respectively, of NNN in smokeless products as other manufacturing practices such as tobacco leaf selection, tobacco curing process, and storage conditions can influence the amount of NNN present in smokeless tobacco products.

The average NNN level for all 34 products was 3.36 μg/g per dry weight. The highest NNN level (12.0 μg/g) was measured for the dry snuff product Dental Sweet. Dry snuff NNN content ranged from 5.91 to 12.0 μg/g per dry weight, with a mean of 7.5 μg/g and a median of 6.0 μg/g. The lowest NNN level (0.635 μg/g per dry weight) was measured for the snus product General Wintergreen. The NNN levels of snus ranged from 0.635 to 2.19 μg/g per dry weight with a mean of 1.3 μg/g and a median of 1.2 μg/g. The NNN levels of chewing tobacco ranged from 0.9 to 4.6 μg/g per dry weight. The average NNN per dry weight was 1.81 μg/g for loose leaf chewing tobacco, 2.2 μg/g for plug chewing tobacco, and 3.1 μg/g for twist chewing tobacco (one twist product had 1.61 μg/g; the other had 4.60 μg/g). The NNN levels of moist snuff ranged from 1.0 to 9.5 μg/g per dry weight, the mean was 3.40 μg/g, and the median was 2.76 μg/g. The NNN value for the CORESTA dry snuff reference product CRP-3 was 7.88 μg/g per wet weight (SD = 0.20 μg/g, 8.66 μg/g per dry weight). This value is, within error, the same as the value of 7.89 μg/g per wet weight reported by CORESTA's Smokeless Tobacco Sub-Group during a 2010 round robin study (see Supplemental Table 3).[23] Although dry snuff products had NNN levels that were consistently high (>5 μg/g per dry weight), NNN levels below 2.5 μg/g per dry weight were measured in smokeless tobacco products that were both relatively wet (water >40%) and relatively dry (water <15%).

Figure 2 compares the NNN levels determined on a wet weight basis to NNN levels on a dry weight basis, grouped by smokeless product type. The data show that the moist snuff products have a larger NNN dry weight to wet weight ratio which most likely reflects the higher water content compared to chewing tobacco, dry snuff, and snus. Interestingly, the moist snuff product that has a lower NNN dry weight to wet weight ratio is Hawken Wintergreen, which has much lower water content (21.4%) than the mean water content of the moist snuff tested (46.4%). The two moist snuff products with the highest NNN content were manufactured by Swisher International Inc. This is in agreement with results published by Borgerding et al., which also showed that Swisher products contain relatively high levels of NNN.[8] Our data show that snus smokeless tobacco products generally have low levels of NNN compared to moist snuff, dry snuff, and chewing tobacco. Furthermore, in comparison to moist snuff, dry snuff, and chewing tobacco, American snus analyzed has comparable NNN levels to the Swedish snus analyzed in this study.

Table 1. NNN and Percent Water Content for 34 Smokeless Tobacco Products Purchased in January 2015[a]

		NNN (μg/g dry wt)		water content (%)	
product name	type	mean	SD	mean	SD
Big Mountain	chewing tobacco, loose leaf	1.01	0.03	19.5	0.3
Bowie Loose Leaf	chewing tobacco, loose leaf	1.40	0.04	21.3	0.6
Bruton Scotch	dry snuff	6.13	0.16	7.91	0.39
Camel Snus Frost	snus, american	1.62	0.09	26.1	0.9
Cannon Ball Plug	chewing tobacco, plug	1.69	0.30	14.8	1.1
Copenhagen Wintergreen, Long Cut	moist snuff	2.40	0.05	51.0	1.0
Copenhagen Original, Fine Cut	moist snuff	3.94	0.20	49.9	1.7
Days O Work Plug	chewing tobacco, plug	2.75	0.16	16.9	1.0
Dental Sweet	dry snuff	12.0	1.9	6.78	0.28
General Wintergreen	snus, swedish	0.635	0.020	44.0	1.3
Grizzly Mint, Long Cut Premium	moist snuff	4.28	0.73	46.9	2.8
Grizzly Straight, Long Cut Premium	moist snuff	3.57	0.12	48.3	0.8
Grizzly Wintergreen, Long Cut Premium	moist snuff	2.60	0.05	46.3	1.8
Hawken Wintergreen	moist snuff	3.51	0.14	21.4	1.0
Husky Wintergreen, Fine Cut	moist snuff	3.44	0.15	49.5	2.1
Kayak Apple, Long Cut	moist snuff	9.50	0.52	48.1	1.2
King B Twist Sweet	chewing tobacco, twist	1.61	0.60	14.9	0.6
Klondike Wintergreen, Long Cut	moist snuff	1.03	0.02	42.5	0.7
Kodiak Wintergreen Premium	moist snuff	2.91	0.35	45.2	1.6
Levi Garrett	chewing tobacco, loose leaf	3.94	0.27	18.4	0.8
Longhorn Wintergreen, Long Cut	moist snuff	2.06	0.04	45.6	3.0
Mammoth Cave Twist	chewing tobacco, twist	4.60	0.52	7.21	0.25
Marlboro Snus Mint	snus, american	0.746	0.027	20.2	0.9
Red Man Golden Blend	chewing tobacco, loose leaf	0.920	0.033	20.7	0.8
Red Seal Natural, Fine Cut	moist snuff	3.03	0.04	48.1	2.9
Silver Creek Cherry	moist snuff	7.45	0.15	47.8	4.5
Skoal Apple Blend, Long Cut	moist snuff	2.05	0.10	48.0	2.8
Skoal Cherry Blend, Long Cut	moist snuff	2.13	0.05	49.6	4.0
Skoal Classic Straight, Long Cut	moist snuff	2.49	0.08	54.8	0.6
Skoal Smooth Mint Snus	snus, american	2.19	0.06	22.3	0.4
Stoker's Wintergreen, Long Cut	moist snuff	2.61	0.10	40.6	0.3
Timber Wolf Straight, Long Cut	moist snuff	2.12	0.12	51.8	2.3
Tops Sweet	dry snuff	5.95	2.07	6.77	0.26
W.E.Garrett & Sons Scotch	dry snuff	5.91	0.17	3.92	0.22

[a]Standard deviation (SD) is provided in addition to average values. All data represent 7 replicates. See Supporting Information for additional details, including product size, manufacturer identification, and flavor.

Figure 3 shows NNN levels as a function of water content for products labeled with a characterizing flavor. Nine products

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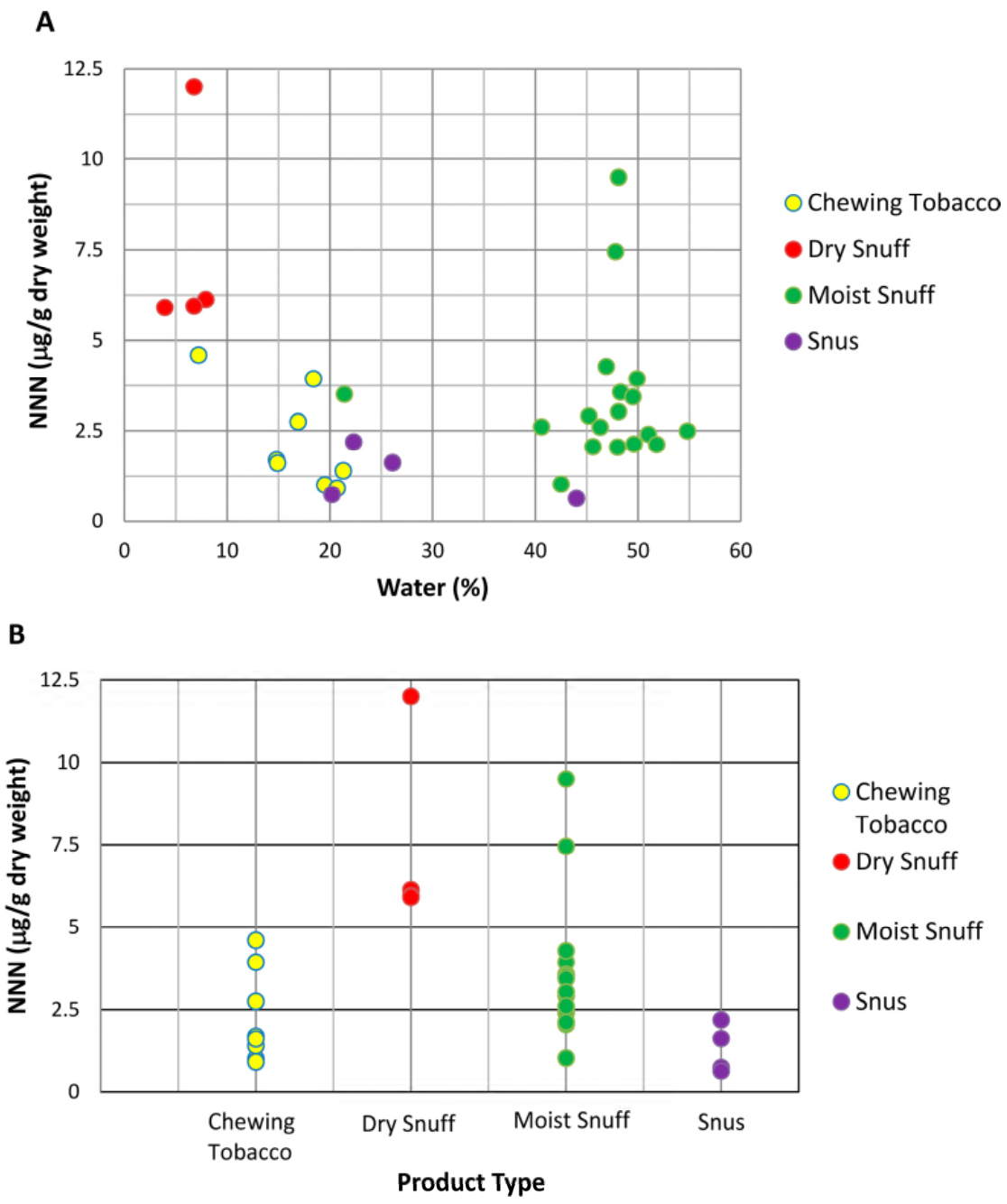

Figure 1. (A) Average NNN levels (μg/g dry weight) as a function of percent water content, with color according to product type. (B) Average NNN levels by product type. The number of products in each column is chewing tobacco = 8 (4 loose, 2 plug, 2 twist); dry snuff = 4; moist snuff = 18; and snus = 4.

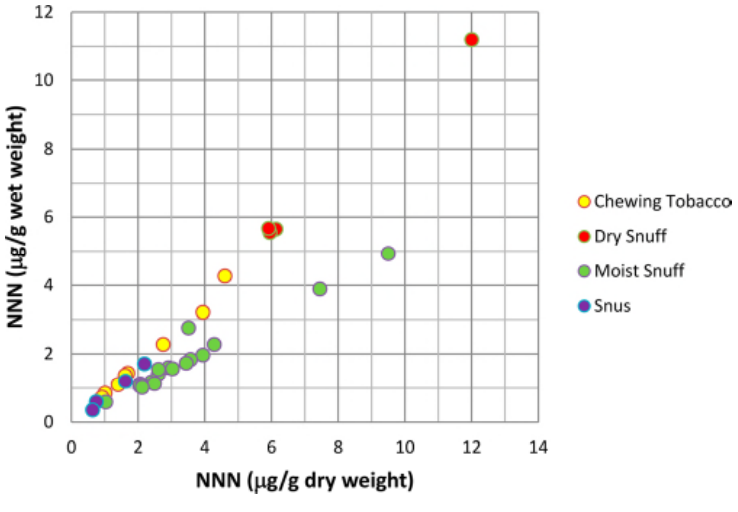

Figure 2. NNN wet weight (μg/g) as a function of NNN dry weight (μg/g), grouped by product type.

with wintergreen flavor, three with mint flavor, two with apple flavor, two with cherry flavor, and one with frost flavor were analyzed. No association is readily apparent between NNN levels and flavor or between water levels and flavor. For example, the moist snuff with the highest NNN level (Kayak Apple Long Cut, 9.5 μg/g per dry weight) and the moist snuff with the lowest NNN level (Klondike Wintergreen Long Cut, 1.03 μg/g per dry weight) have characterizing flavors.

Water Content Results. There are various methods for measuring the total water content of smokeless tobacco. AOAC Official Method 966.02[24] involves weighing a five gram tobacco sample, placing the sample in a forced-draft drying oven set at 99.5 °C ± 0.5 °C for 3 h, and calculating the water using weight-by-difference. However, Bourlas et al.[18] have shown that tobacco products that have been oven-dried not only liberate volatiles other than water but also lose water via the degradation of organic compounds such as acetic acid and reducing sugars, yielding higher values than the true water content. Other methods have been developed to determine

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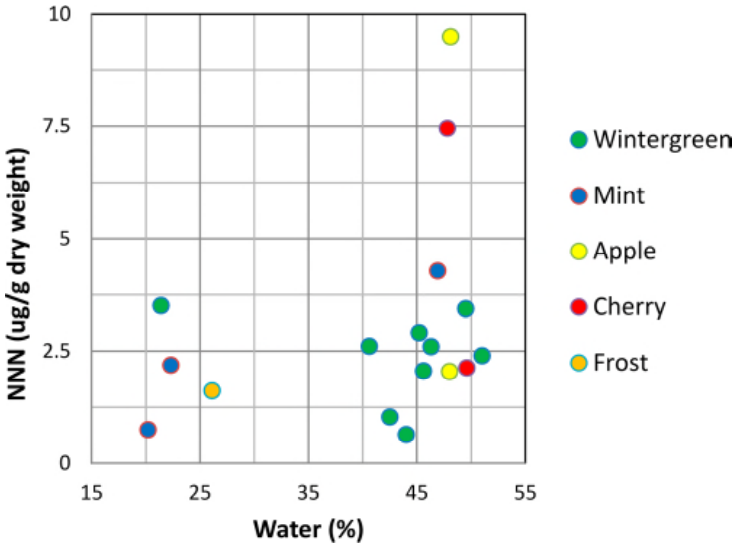

Figure 3. NNN (μg/g dry weight) for flavored smokeless tobacco products as a function of water content.

water content in tobacco products. CORESTA methods No. 56[25] and 57[26] describe the determination of water in tobacco and tobacco products by the Karl Fischer method and gas chromatography (GC) analysis, respectively. Likewise, ISO methods 6488[27] and 16632:2013[21] also describe the Karl Fischer method and GC analysis, respectively. Richter and Spierto[28] used the AOAC oven volatiles method to assay the water content of 18 brands of smokeless tobacco. In our current study, water was quantified by gas chromatography according to ISO method 16632:2013. Water content results are reported in Table 1 and shown in Figure 1A, Figure 3, and Table 3. The product with the highest water content was a moist snuff product with 54.8% water content. Moist snuff water content ranged from 21.4 to 54.8%, the mean was 46.4%, and the median was 48.1%. The driest product was a dry snuff product with 3.9% water content. Dry snuff water levels ranged from 3.9% to 7.9%, the mean was 6.3%, and the median was 6.8%. Chewing tobacco moisture levels ranged from 7.2 to 21.3%. The average water content was 20.0% for loose leaf chewing tobacco, 15.8% for plug chewing tobacco, and 11.0% for twist chewing tobacco (one twist product was 7.2%; one was 14.9%). Snus water levels ranged from 20.2 to 44.0%, the mean was 28.1%, and the median was 24.2%.

Comparison with Previously Published NNN Market Surveys. Table 2 compares NNN levels for 13 smokeless tobacco products that were analyzed both in the current study and in earlier studies (see also Supplemental Figure 1).[6,7,9,11,12] All NNN levels in Table 2 were presented in wet weight in order to make direct comparisons. The NNN levels show no general increasing or decreasing trends for the 12 common products that were analyzed in the 2006–2007 study compared to our current 2015 study: seven products in the 2006–2007 study showed a decrease in NNN levels in 2015 while five products showed an increase in NNN levels. However, Figure 4 shows a general *decrease* in NNN levels for all six moist snuff products that were analyzed in 2004, 2006–2007, and our current 2015 study. Although the total water content was determined by different methods (the oven volatiles method in 2004 and the GC method in 2015), the large decreases in NNN levels for the six moist snuff products (average 68% decrease) are most likely not attributed to differences in the two water content methods. Table 2 shows that the NNN levels in two loose-leaf smokeless products (Levi Garrett and Red Man

Table 2. Comparison of NNN (μg/g wet weight) in Smokeless Tobacco Products from Six Studies[a]

		NNN, μg/g wet wt													
		2004[7]			2006–2007[9]			2007–2009[11]			2010[12]			2011[6]		2015 (current work)		
name	type	mean	SD	N	mean	SD	N	mean	SD	N	mean	SD	N	mean	SD	N	mean	SD	N
Grizzly Wintergreen, Long Cut Premium	moist snuff	3.633	0.25	3	1.614	0.030	3										1.40	0.03	7
Hawken Wintergreen	moist snuff	3.203	0.19	3	2.026	0.088	3										2.76	0.11	7
Kodiak Wintergreen Premium	moist snuff	6.923	1.674	3	2.307	0.093	3										1.60	0.19	7
Red Seal Natural, Fine Cut	moist snuff	14.08	4.472	3	1.953	0.026	3										1.57	0.02	7
Skoal Cherry Blend, Long Cut	moist snuff	6.237	1.368	3	1.982	0.039	3										1.07	0.03	7
Skoal Classic Straight, Long Cut	moist snuff	8.186	0.411	3	2.156	0.087	3										1.13	0.04	7
Longhorn Wintergreen, Long Cut	moist snuff				1.402	0.010	3										1.12	0.02	7
Days O Work	chewing tobacco																2.28	0.13	7
Red Man Golden Blend	chewing tobacco				0.493	0.023	3										0.729	0.026	7
Levi Garrett	chewing tobacco				2.227	0.076	3										3.21	0.22	7
Bruton Scotch	dry snuff				5.164	0.035	3										5.65	0.15	7
Dental Sweet Snuff	dry snuff				13.126	0.099	3										11.2	1.8	7
Camel Snus Frost	snus				0.684	0.026	3	2.92	0.9	3	0.36	0.04	17	1.33	0.56	17	1.20	0.07	7
Camel Snus Spearmint	snus							0.942	0.022	3									
Camel Snus Original	snus							0.425	0.053	3									

[a]Year indicates the purchase date for products analyzed in the study. To improve the comparison, datasets from any study for which NNN was reported as dry weight were converted to wet weight using the water content reported in that study.

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Table 3. Comparison of Percent Water Content in Smokeless Tobacco Products from Four Studies, Measured as Oven Volatiles (OV) Using a Gravimetric Method, or Measured by Gas Chromatography with Thermal Conductivity Detection (GC-TCD)[a]

		water content (%)											
		2006–2007, OV[9]			2010, OV[12]			2011, OV[6]			2015, GC-TCD (current work)		
product name	type	mean	SD	*N*	mean	SD	*N*	mean	SD	*N*	mean	SD	*N*
Grizzly Wintergreen, Long Cut Premium	moist snuff	53.2	0.1	3							46.3	1.8	7
Hawken Wintergreen	moist snuff	29	0	3							21.4	1.0	7
Kodiak Wintergreen Premium	moist snuff	54.2	0.1	3							45.2	1.6	7
Red Seal Natural, Fine Cut	moist snuff	54.6	0.1	3							48.1	2.9	7
Skoal Cherry, Long Cut	moist snuff	53.7	0.1	3							49.6	4.0	7
Skoal Classic Straight, Long Cut	moist snuff	54.4	0	3							54.8	0.6	7
Longhorn Wintergreen, Long Cut	moist snuff	54.7	0.1	3							45.6	3.0	7
Red Man Golden Blend	chewing tobacco	25.5	0.1	3							20.7	0.8	7
Levi Garrett	chewing tobacco	21.6	0.2	3							18.4	0.8	7
Bruton Scotch	dry snuff	7.2	0.1	3							7.91	0.39	7
Dental Sweet Snuff	dry suff	9	0.1	3							6.78	0.28	7
Camel Snus Frost	snus	32.2	0.1	3	29.2	3.4	17	32.8	1.5	17	26.1	0.9	7

[a]The year is the purchase date for products analyzed in the study.

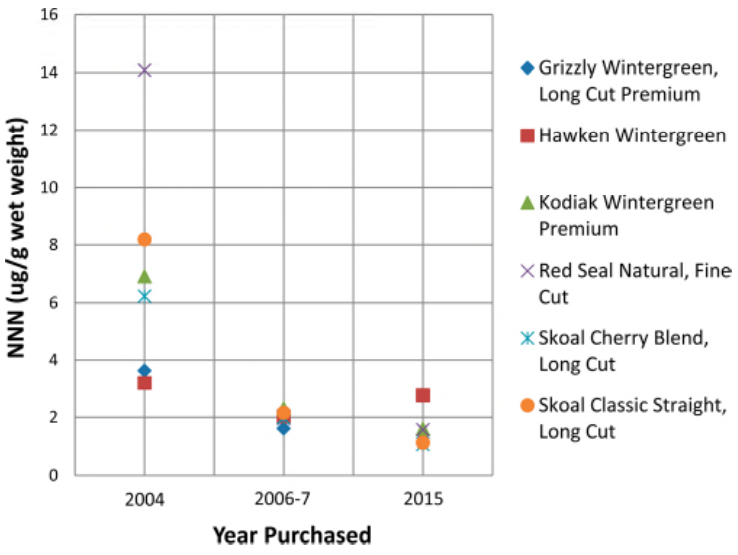

Figure 4. NNN (μg/g wet weight) levels for moist snuff products that were purchased and analyzed in three surveys from 2004 to 2015.

Golden Blend) increased upon comparison of the 2006–2007 study with our current 2015 study; however, the small data set warrants no general conclusion for loose leaf smokeless tobacco products.

Comparison of Previously Published Water Content Market Surveys. The data set for water content is compared to data sets from three other studies in Table 3. Of the 12 products for which a comparison could be made, water levels measured as oven volatiles using a gravimetric method on products purchased in 2006–2007 are on average 17% higher than water levels measured by GC-TCD on products purchased in 2015. Because the gravimetric method is a weight-by-difference method and defines water content as weight loss after 3 h at 100 °C, it yields higher values for water than the GC-TCD method, which measures only water content. Interestingly, the water content of the CORESTA dry snuff reference product CRP-3 was found to be 9.00%. This is higher than the water content of 8.206%, measured as the weight of oven volatiles determined by CORESTA for this reference product during the 2010 round robin study.[23] The apparent increase in water content of the CRP-3 reference material using the GC-TCD is most likely due to the relatively large standard deviation of the dry snuff reference (SD = 1.39) and may not be statistically significant. The use of different methods and, to some degree, product composition changes may be contributing factors to the differences in water content between the 2006–2007 and 2015 studies.

Our analysis of NNN and water levels in 34 smokeless tobacco products purchased in the United States in 2015 produced a snapshot of NNN levels in currently available products and allowed comparison of product types that have different water levels. The NNN levels show no general increasing or decreasing trends for the 12 common products that were analyzed in the 2006–2007 study compared to our current 2015 study. We observed a general decrease in NNN levels for all six moist snuff products that were analyzed in the 2004 samples compared to our current 2015 study. In contrast to other available market studies, we report moisture as water content as opposed to weight of oven volatiles, which eliminates sources of error including the loss of volatile flavors or loss of volatile organics formed by decomposition of sample components during the oven heating step. We observed that water levels measured as oven volatiles on products purchased in 2006–2007 were on average 17% higher than water levels measured by GC-TCD in 2015. It is not clear whether this difference is due to differences in water content of the analyzed products in each study, due to differences in the analytical method, or both. We observed that characterizing flavors, including wintergreen, apple, cherry, mint, and frost, had no impact on the NNN and water levels of smokeless tobacco products that were labeled as characterizing flavors. We observed that the levels of NNN (μg/g dry weight) in moist snuff have decreased since 2004. Our data show that snus smokeless tobacco products generally had lower levels of NNN. Our data also show that, when compared to moist snuff, dry snuff, and chewing tobacco, American snus analyzed had NNN levels comparable to those of the Swedish snus analyzed in this study.

■ ASSOCIATED CONTENT

Supporting Information

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Table of NNN, percent water content, and product identifying information for smokeless tobacco products, plot of NNN wet weight levels by product purchase date, and label and purchase information (PDF)

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Notes

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